Physiology and Pathophysiology of Proteinase-Activated Receptors (PARs): PARs in the Respiratory System: Cellular Signaling and Physiological/Pathological Roles

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Abstract. Proteinase-activated receptors (PARs), a family of G protein-coupled receptors, are widely distributed in the mammalian body, playing a variety of physiological/pathophysiological roles. In the respiratory systems, PARs, particularly PAR-2 and PAR-1, are expressed in the epithelial and smooth muscle cells. In addition to the $G_{q/11}$-mediated activation of the phospholipase $C_\beta$ pathway, epithelial PAR activation causes prompt and/or delayed prostanoid formation, leading to airway smooth muscle relaxation and/or modulation of an inflammatory process. PAR-2 present in the epithelium and smooth muscle is considered primarily pro-inflammatory in the respiratory system, although PAR-2 may also be anti-inflammatory under certain conditions. In the lung epithelial cells, PAR-2 can also be activated by exogenous proteinases including house dust mite allergens, in addition to various possible endogenous agonist proteinases. Clinical evidence also suggests possible involvement of PARs, particularly PAR-2, in respiratory diseases. PARs thus appear to play critical roles in the respiratory systems, and the agonists/antagonists for PARs may serve as the novel therapeutic strategy for treatment of certain respiratory diseases including asthma.

Keywords: proteinase-activated receptor, proteinase, airway, inflammation, smooth muscle relaxation

Introduction

Proteinase-activated receptors (PARs) are a family of G-protein-coupled seven-trans-membrane-domain receptors, consisting of four members, PARs 1 to 4 (1–5). Thrombin is an endogenous agonist for PAR-1, PAR-3, and PAR-4. Receptor activation by thrombin is achieved by unmasking the N-terminal cryptic receptor-activating sequence of each receptor (e.g., SFLLR---- for human PAR-1) that binds to the body of the receptor as a tethered ligand. PAR-2 is activated in a similar fashion by trypsin, trypase, and coagulation factors VIIa and Xa, but not thrombin. Exogenously applied synthetic peptides as short as 5–6 amino acids based on the sequence of tethered ligands (e.g., SFLLR, SLIGKV, and GYPGQV for human PAR-1, PAR-2, and PAR-4, respectively) are also capable of activating PARs by directly binding to the body of the receptors, although non-proteolytic activation of PAR-3 is unknown (6).

PARs, especially PAR-1 and PAR-2, are expressed extensively in various tissues and cells throughout the mammalian body. A variety of physiological/pathological roles for PARs have been described in the respiratory (7–9), gastrointestinal (10–12), circulatory (13, 14), and nervous systems (15–17) and also in the skin (6). Here we focus on cellular signaling triggered by PARs and their functions in the respiratory system. Physiological/pathological functions of PARs in other systems have been well-reviewed elsewhere (6, 12, 14, 17, 18).

Cellular signaling triggered by PARs in various tissues/cells, particularly in the respiratory system

The best known common signaling pathway follow-
ing activation of PARs includes activation of phospholipase Cβ via Gq/11 protein, which produces inositol triphosphate followed by Ca2+ mobilization and diacylglycerol followed by activation of protein kinase C (6). However, other multiple signaling pathways have also been described for PARs, particularly PAR-1 and PAR-2. PAR-1 also interacts with multiple α-subunits of Gα proteins other than Gq/11α, in particular, G12/13α and G13α. G12/13α coupled to PAR-1 interacts with Rho guanine-nucleotide exchange factors (GRFs) in platelets and astrocytoma cells, leading to Rho-mediated control of cell shape and migration (19). PAR-1 also triggers activation of the mitogen-activated protein (MAP) kinase cascades and is capable of trans-activating epidermal growth factor (EGF) receptors (6, 20). The PAR-2-triggered signaling mechanisms other than the Gq/11α-mediated activation of phospholipase Cβ have yet to be analyzed in detail. In various cells/tissues, PAR-2 activation causes arachidonic acid release and prostanoid formation (21) and stimulates the MAP kinase cascades (6, 22, 23). There is also evidence that trans-activation of EGF receptors is involved in PAR-2-facilitated cell growth in colon cancer cells (23).

In the respiratory system, PAR-2 and PAR-1 are expressed in both the epithelial and smooth muscle cells (6, 8). In addition to possible activation of Gq/11α-mediated activation of phospholipase Cβ (24, 25), epithelial PAR-2 stimulation causes prompt prostanoid formation in isolated airway tissues (26) and evokes delayed accumulation of prostanoids in human respiratory epithelial cells (27, 28). The delayed prostanoid formation in the cultured epithelial cells appears to involve cyclooxygenase (COX)-2 induction via multiple signaling pathways including the MAP kinase cascades (28). Although PARs play extensive roles in the respiratory systems, other signal transduction mechanisms for PARs in the airway epithelial and smooth muscle cells are still largely open to question.

Modulation of smooth muscle motility and ion transport by proteinases and PARs in the airway

Cocks and Moffatt (8) have shown that in the airway tissues isolated from mice, rats, guinea pigs, and humans, PAR-2 and PAR-1 are present in the epithelial cells and, upon activation, cause prostanoid-dependent smooth muscle relaxation. They have also detected immunoreactive trypsinogen/trypsin, as a possible endogenous activator for PAR-2, in the airway epithelium. Lan et al. (26) have actually identified prostaglandin E2 release from mouse tracheal tissues. Ultimate evidence for involvement of PAR-2 in the PAR-2-activating peptide-evoked airway relaxation has been obtained in the experiments using PAR-2-knockout mice (9). PAR-1 and/or PAR-2 are also present in the airway smooth muscle or lung parenchyma and, upon activation, may elicit contractile responses in distinct species (29–31).

Agonist proteinases for PARs including trypsin and thrombin are not necessarily as potent as their receptor-activating peptides in modulation of the airway smooth muscle tone (9, 29). Neither trypsin nor thrombin at 0.01–10 U/ml modifies the tension in the smooth muscle segments of rat trachea, main and first order intrapulmonary bronchi. In contrast, PAR-2- and PAR-4-activating peptides exhibit potent relaxant activity, and PAR-1-activating peptides cause contraction in the airway preparations from rats (29). Interestingly, trypsin is a potent relaxant in mouse bronchus, but much less potent as a relaxant in mouse trachea (9). No activity or poor potency of trypsin as a relaxant in rat airway and mouse trachea as described above is attributable to high levels of expression of endogenous proteinase inhibitors in these tissues, although no direct evidence is available. However, the physiological relevance of the distinct potency of trypsin in different species and regions remains to be interpreted. The potency of trypsin as a tracheal and bronchial relaxant decreases but does not disappear in PAR-2-knockout mice. In addition, desensitization of both PAR-2 and PAR-1 does not abolish the relaxant effect of trypsin in the airway (9). Therefore, trypsin-evoked airway relaxation appears to involve both PAR-2-dependent and -independent mechanisms. PAR-4 could contribute, at least in part, to the PAR-2-independent component of the effect of trypsin in the airway (9).

PAR-2 and/or PAR-1 modulate ion transport in the intestine (32–35) and pancreatic duct (36). Similarly, PAR-2 regulates electrolyte transport in the airway epithelium, although there is no evidence for PAR-1 regulation of ion transport in the respiratory system (37, 38). PAR-2 activators, when added to the basolateral, but not apical, surface of airway epithelial cells, regulate Na+ absorption and Cl− secretion in a cytosolic Ca2+-dependent manner (37).

Roles of PARs in airway inflammation

Epithelial PAR-2 might play a protective role in the airway in certain conditions, since PAR-2 agonists cause epithelial prostanoid-dependent bronchodilation as described above and also suppress some inflammatory responses, such as bacterial lipopolysaccharide-induced recruitment of polymorphonuclear leukocytes into mouse airway (8, 39) and histamine-induced enhancement of vascular permeability in guinea pig airway (40). Nonetheless, there is abundant evidence for pro-inflam-
flammatory roles for PARs, particularly PAR-2. In the human alveolar epithelial cells (A549), activation of PAR-2 induces enhancement of expression and/or release of matrix metalloproteinase-9 (MMP-9) and granulocyte macrophage-colony stimulating factor (GM-CSF), important mediators of inflammation (41, 42). Release of interleukin (IL)-6, IL-8, and prostaglandin E2 also occurs following PAR-2 and/or PAR-1 stimulation in A549 cells and also primary human bronchial epithelial cells (27, 28). There is in vivo evidence that intranasal administration of a PAR-2 agonist stimulates macrophage infiltration into bronchoalveolar lavage fluid in mice. Furthermore, ovalbumin (OVA) challenge of OVA-sensitized wild-type mice induces leukocyte infiltration into bronchoalveolar lavage and airway hyperreactivity to inhaled methacholine, and these inflammatory responses to OVA challenge are inhibited in mice lacking PAR-2 and increased in mice overexpressing PAR-2, compared with wild-type animals (43). In the airway smooth muscle, PAR-2 and/or PAR-1 are present and regulate DNA synthesis and mitogenesis/proliferation (44 – 46). Mast cell tryptase, a possible endogenous activator for PAR-2, also stimulates smooth muscle cell proliferation, although some differential characteristics have been detected in the effects of tryptase and PAR-2-activating peptides on proliferation (44, 46, 47). Collectively, PAR-2 mediates smooth muscle proliferation, which, in addition to the pro-inflammatory roles of PAR-2 in the epithelium, may contribute to development of airway diseases such as asthma.

Tryptase, tryptase, and coagulation factors VIIa and Xa are considered possible endogenous activators for PAR-2 in the airway (6), although additional novel candidates for PAR-2 agonists including trypsin IV (48), neutrophil protease 3 (49), and membrane-type serine protease-1 (50) have been reported. Most interestingly, house dust mite allergens, such as Der p1, Der p3, and Der p9, are capable of activating PAR-2 and stimulating release of pro-inflammatory cytokines including GM-CSF, eotaxin, IL-6, and IL-8 in human lung epithelial cells, although Der p1 might inactivate PAR-1 (51, 52). These findings suggest that mite allergens may induce a non-allergic airway inflammation through the release of pro-inflammatory cytokines by activating PAR-2.

Clinical evidence for roles of PARs in pathogenesis of airway diseases

Miotto et al. (53) have reported that an increased expression of PAR-2 was found in bronchial vessels of smoker patients with bronchitis compared with those with chronic obstructive pulmonary disease (COPD), although PAR-2 expression was similar in smokers and non-smokers. Knight et al. (54) have shown that asthma per se is associated with increased immunostaining for PAR-2, but not PAR-1, PAR-3, and PAR-4, in bronchial epithelium, which is not influenced by inhaled corticosteroids. On the other hand, Chambers et al. (55) have reported that trypsin, but not PAR-2-activating peptides or tryptase, caused prostaglandin E2 release and induction of COX-2 at mRNA and protein levels in airway

![Fig. 1. A scheme for possible roles of PAR-2 in the respiratory system. F VIIa and F Xa, coagulation factors VIIa and Xa, respectively; PGE2, prostaglandin E2; EP2, prostaglandin EP2 receptor. Tryptase from mast cells, factors VIIa and Xa from blood vessels, and possibly trypsin from the adjacent epithelial cells activate PAR-2 in the epithelium, which in turn causes release of various inflammatory mediators and promotes inflammation. Mite antigens, Der p1, Der p3, and Der p9, could be exogenous agonists for PAR-2. Furthermore, epithelial PAR-2 activation may protect against inflammation under certain conditions, producing prostaglandin E2, which causes airway smooth muscle relaxation. On the other hand, activation of PAR-2 in the airway smooth muscle is capable of facilitating proliferation.](image-url)
smooth muscle cells isolated from asthmatic patients, the former effect being less potent than that in nonasthmatic airway epithelial cells. Thus, PAR-2-activating proteinases and/or PAR-2 might participate in the pathogenesis of human airway diseases.

Conclusion

PARs, particularly PAR-2, thus appear to play critical roles in the respiratory systems, particularly under pathological conditions. Of interest is that PAR-2 plays a dual role in inflammation, which might be anti-inflammatory in an acute phase and pro-inflammatory in a delayed phase during the development of inflammation (8, 56). A hypothetical scheme for roles of PAR-2 in the respiratory system is shown in Fig. 1. Taken together, PAR-2 antagonists, if any, might serve as therapeutic drugs for certain respiratory diseases such as asthma and COPD, although PAR-2 agonists might also have some therapeutic benefit for certain respiratory diseases.

References


